Development Of Some New Chromogenic Spray Reagents For Thin Layer Chromatography And Their Applications In Analysis Of Toxic Materials

RESEARCH ABSTRACT

(Proposed Work Plan for Ph.D.)

Submitted to

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PROPOSAL FOR Ph.D. REGISTRATION

Proposed Research Work

Title:

Development of Some New Chromogenic Spray Reagents For Thin Layer Chromatography And Their Applications In Analysis Of Toxic Materials

1. Introduction:

The use of organophosphorus insecticides has been greatly increased after ban on many Organochloro pesticides and insecticides. Many of the agricultural products are reported to have higher pesticide concentration than prescribed limit. The misuse of these easily available chemicals has been increased in same manner especially in cases of farmer suicide in India[1]. Although there are many instrumental techniques for qualitative and quantitative analysis of pesticides, drugs viz. HPLC, IR, NMR the Thin Layer Chromatography is still method of choice for complex matrix like visceral tissue, due to fast analysis, cost effective nature. So there is need to identify such toxic materials such as pesticides and drugs from post mortem visceral tissues in crime laboratory as well as from commercial formulations of pesticides and drugs using Thin Layer Chromatography.

2. Review of Literature and Development in the Subject:

The origin of thin layer chromatography is a little obscure but was probably first developed and utilized by Schraiber[2] in 1939. Schraiber working with Izmailov at the Khar'kov Chemistry and Pharmacy Research Institute developed the techniques for use in the analysis of pharmaceuticals. Quoting from her publication,

" *It occurred to us that a thin layer of the sorbent could be used in lieu of a strip of paper; also we felt that the flat bed could be considered as a cut-out of the adsorbent column. We believed that in carrying out the separation process in such a layer, the process would be accelerated significantly. In our work, we deposited a drop of the solution being investigated on the flat adsorbent layer and observed the separation into concentric circular zones which could become visible because of their fluorescence in the light of a UV lamp.*"

Today TLC is used for separation, identification of complex mixtures and also as a pilot method for column chromatography.

. It was only in 1954, TLC was first used for the determination of pesticide residues when Kirchner and Rice analysed biphenyl in citrus fruits using silica gel chromato-strips.The use of TLC in analysis of pesticide was reviewed by Stahl[3] In 1956 who invented an automatic spreader for TLC plates. Many research reported and publications started appearing in later years dealing with TLC, for carbamates by Fishbein and TLC of organophosphorus compounds by Sherma are a few examples. In later years many visualization reagent, also called as chromogenic spray reagents were discovered by some Indian researcher. Analysis of many nitro pesticides is based on reduction of nitro groups in pesticide molecule to amino group and then making a coloured dye by subsequent attack by a suitable nucleophile on it, or using a derivative of pesticide as a nucleophile for attack on suitable diazo compound. The analysis of such insecticides from post mortem tissues and body fluids is essential in forensic analysis. Many such attempts has been successful not only for qualitative analysis but also for quantitative analysis.

In 2006, P. Nagaraja developed Dapsone as a chromogenic spray reagent for carbamate insecticide, carbofuran[4].Chandegaokar identified Monocrotophos using Benzil as a new chromogenic spray reagent[5] in 2009. Carbosulfan was detected in 2010 by K.V. Kulkarni[6] using Potassium Ferricyanide. Anthranilic Acid was shown to be useful as a chromogenic spray reagent for Chlorpyrifos by N.V.S. Venugopalan[7] in 2012...N.L. Chutke[8] was able to simultaneously analyze many pesticides using single spray in 2013.Profenofos was identified in Biological Samples by Lanjewar[9] using o-Toluidine in 2014. Fenitrothion was detected by a chromogenic spray reagents by S.S. Prabhavale[10] usingiminodibenzyl as a nucleophile in 2015.

Dichlorvos was analyzed by Mr. B. B. Daundkar using sodium sulphide to obtain yellow spots of insecticide. The work in this field is progressing and many new reagents are being developed, some being non-specific and some being specific. Their sensitivity varies according to insecticide. But still there are many Insecticides whose specific sprays are unknown. Some pesticides doesn’t possess any special functional group hence their identification using this technique is tricky. Analysis of pesticide residue from post mortem tissues and body fluid is challenge as many interfering compounds are present eg. Phenolic bodies in tissue cause false positive results when testing phenolic insecicides. Hence the work in this fieldhas good scope.

3. Research Objectives:

The objectives of the proposed study are:

- To prepare some new Chromogenic Spray Reagent for different toxic materials like some Pesticides, Insecticides and drugs
- To develop methods and new solvent system for efficient extraction of such chemicals from commercial formulations, spiked samples, visceral tissues, body fluids, agro products and environmental samples.
- To evaluate these new chromogenic spray reagent for qualitative and semi quantitative analysis using Thin layer Chromatography and spectrophotometry.

4. Methodology to be adopted:

- First literature survey will be done for available Chromogenic spray reagents for different available toxic materials (pesticides, drugs etc.)
- Based on type of toxic material such as drug, pesticide (Organophosphorus, Organochloro, Pyrethroid etc.) and its structure, new reagents (Organic or Inorganic or Organometallic) will be tested which may give colour reaction with toxic materials of interest.
- The optimum solvent system or its combinations and extracting solvent will be found for given compound.
- From multiple reagents found so far, the more sensitive, more specific, cheaper and more efficient reagent will be sorted out and will be taken for further studies.
- The mechanism of refaction for formation of the coloured product will be studied.
- The instrumental Analysis for the given compound will be performed with spectrophotometer.
- Results, Discussion and Analysis (Conclusion)

- **Significance of the Study**

Today, thousands of different commercial formulations of toxic materials like drugs and pesticides are available and many new being prepared every year. Pesticides are now a part of our agricultural system. Even though the organic farming is current trend, the Organic food costs much more than the normal Agro products. In many places, there is no monitoring system for quantity of pesticide to be used in farm. Hence now, pesticides traces are being found in many agro products like fruits, vegetables and even in soil samples. As many pesticide are potential carcinogenic, some of them cause high

environmental, soil, water pollution (eg. chlorinated pesticide) and many being used for suicidal attempt due to easy availability and lack of monitoring system, the proper identification method for them is necessary from pure sample as well as traces in post-mortem tissues and soil, water etc.

There are still many toxic materials (pesticides, drugs etc) whose standard identificationis based mainly on Instrumental Techniques like HPLC. These techniques even though being sensitive, are not available in small budget laboratories. Thin layer chromatography has been proved very efficient, pocket friendly and easy to use techniques for such samples. It can also be used to pre-analyse different toxic materials before using Instrumental Techniques as a pilot method. Also extraction of toxic materials traces from visceral tissues and body fluid required several cleaning and other sample preparation steps for instrumental analysis, which is not required by TLC. Many sample can be analysed simultaneously. Hence TLC can be used effectively for identification of many toxic materials. To detect the analyte on TLC, some techniques like irradiation of plate with UV rays, use of fluorogenic reagents etc. has been used. However to use chromogenic reagent is best for those analytes which can form coloured derivatives, intermediates or products, as it gives direct result visible to naked eyes. Many of the toxic materials fits in these criteria as most of them are organic compounds whose colored derivative can be prepared based on their structure and available or convertible functional group.

6.Proposed Work Plan:

1st Year

- Literature survey (2 months)
- Development of optimum solvent system for given toxic materials (insecicides, pesticides drugs etc)
- Development of some new chromogenic spray reagents for given toxic materials (insecicides, pesticides drugs etc)

2nd Year

- Study of mechanism of reaction for formation of coloured product.
- Optimization of reaction under study for pH and other reaction condition.
- Communication of research articles to reputed journals.

3rd Year

- To perform instrumental (Spectrophotometric) analysis of given insecticides.

- Communication of research articles to reputed journals
- Submission of Synopsis.

PROPOSED SCHEMES

1.) Development of some new chromogenic spray reagents for **Organophosphorus** Insecticides.
2.) Development of some new chromogenic spray reagents for **Organochloro** Insecticides
3.) Development of some new chromogenic spray reagents for **Pyrethroid** Insecticides
4.) Development of some new chromogenic spray reagents containing **miscellaneous functional groups**
5.) Development of some new chromogenic spray reagents for some **drugs**

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